Early development of the endangered Oxleyan pygmy perch *Nannoperca oxleyana* Whitley (Percichthyidae)

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ABSTRACT

The developmental ontogeny and morphology of the eggs, larvae and early juveniles of the endangered *Nannoperca oxleyana* is described based on collections of preserved wild fish, and preserved and live captive specimens reared at 25 ± 1° C. Eggs are telolecithal, spherical, average 1.02 ± 0.004 (± S.E.) mm in diameter, have a smooth, slightly adhesive chorion without filaments, a translucent homogeneous yolk, display meroblastic cell division and follow the general pattern of teleost embryogenesis. Early, middle and late stages of embryonic development were completed on average at 16, 28 and 50 hours post fertilisation. The larvae have generalised perciform morphological development with no apparent larval specialisations. The body is moderately deep bodied and compressed laterally. Head spination is limited to the development of an opercular spine in postflexion larvae. Pigmentation is relatively heavy and uniform over the head and body. Large melanophores occur on the dorsal, lateral and ventral midlines. Squamation commenced in postflexion larvae from 7.5 mm preserved body length (BL) and was complete in all specimens greater than 10.3 mm. Live captive, translucent larvae become pale green within two days of hatching and then light brown during the preflexion and postflexion stages. Juveniles are typically light brown laterally, darker dorsally and have a silvery-white belly. Growth of live, captive larvae and juveniles up to 6-months post hatching was described by the equation: Log_{10} live BL = 0.6043 + 0.0042A ($r^2 = 0.917$, $P<0.001$, $n = 323$). Ranges for body length and age (days post hatch) of live captive fish were: preflexion 2.80-6.70 mm, 0-40 days; flexion: 6.35-7.70 mm, 21-61 days; postflexion: 6.40-10.30 mm, 43-118 days; and juveniles: 9.60-20.60 mm, 70-188 days. Live captive larvae commenced exogenous feeding at five days post hatching. Comparisons of *N. oxleyana* early developmental ontogeny and morphology are made with related percichthyids and sympatric species and implications for the conservation of the species are discussed.

Key words: *Nannoperca*, embryology, larval development, ontogeny.

Introduction

Pygmy perch are a group of small, freshwater fishes endemic to southern Australia. The three genera of pygmy perch *Edelia*, *Nannatherina* and *Nannoperca* are considered members of the family Percichthyidae (previously Nannopercidae) (Hoese *et al.* 2006). Recent phylogenetic analysis supports the placement of the two species of *Edelia* (*E. vittata* and *E. obscura*) within *Nannoperca* (Jerry *et al.* 2001), which we follow here (although see Hoese *et al.* 2006). *Nannoperca oxleyana* Whitley inhabits coastal northern New South Wales (NSW) and southern Queensland (Qld), and represents the northeastern distribution limit of these genera (Kuiter and Allen 1986; Kuiter *et al.* 1996). *Nannatherina balstoni* Regan and *Nannoperca vittata* (Castelnau) are restricted to southwestern Australia, while *Nannoperca variegata* Kuiter and Allen, *Nannoperca obscura* (Klunzinger) and *Nannoperca australis* Günther are endemic to the southeastern corner of the country. Cryptic speciation exists with *N. vittata* and *N. australis* each apparently comprised of two distinct, yet undescribed species (Hammer 2002; M. Hammer, University of Adelaide, South Australia, pers. comm.).

Pygmy perch are amongst Australia's most threatened fauna with four of the five described species threatened with extinction. Many populations have been lost or are under threat from habitat degradation and negative interactions with exotic species. A number of management plans have been developed with the aim of assisting these species to recover to a position of viability in nature (Fisher 1993; Arthington 1996; Hammer 2002; NSW Department of Primary Industries 2005). Recovery planning requires knowledge of the biology of a species to mitigate human impacts. Detailed biological data are therefore vital prerequisites in planning and implementing effective conservation management programs.

Although a number of studies have examined various aspects of the biology of pygmy perch, for example, habitat associations (Bond and Lake 2003; Knight and Arthington 2008), reproduction (Humphries 1995; Knight *et al.* 2007), diet and growth (Pen and Potter 1991; Morgan *et al.* 1995; Arthington 1996), breeding in captivity (Llewellyn 1974; Wager 1992; Briggs 1999; Knight *et al.* 2007), and population genetics (Hughes *et al.* 1999; Hammer 2001; Knight *et al.* 2009), there remains a lack of research into their early life histories. Studies of the eggs and larvae of pygmy perch are restricted to incomplete descriptions for *N. vittata* (Shipway 1949; Morgan and Beatty 2000) and *N. australis* (Llewellyn 1974), and a more comprehensive analysis of the larval development and diet of *N. balstoni* (Gill and Morgan 1998). This is indicative of the paucity of literature on Australian percichthyids, with the larvae of only six of 15 species fully described (Gill and Morgan 1998; Leis and Trnski 2004; Trnski *et al.* 2005).

Nannoperca oxleyana is recognised as the most threatened pygmy perch and is classified as endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) and under Australian Commonwealth and State legislation (NSW Department of Primary Industries 2005). This species obtains a length of approximately 60 mm, is a microphagic carnivore, and inhabits shallow, swampy regions of dystrophic, acidic streams, lakes and swamps within coastal 'wallum' (*Banksia* dominated heath) ecosystems (Kuiter *et al.* 1996; Knight 2000; Pusey *et al.* 2004; Knight and Arthington 2008). Protracted serial spawning occurs during the warmer months of the year between September and May when mean day length and water temperature reach and exceed 10.7 hours and 16.6° C, respectively (Knight *et al.* 2007). Despite a lack of research into the species' early life history, aquarium enthusiasts have reported eggs hatching in one to four days at temperatures greater than 20° C (Leggett and Merrick 1987; Wager 1992). Larvae commenced feeding one to two days post hatching (Wager 1992) and grew to a total length of 18 mm within 10 weeks (Leggett 1990).

This study describes the developmental ontogeny and morphology of the eggs, larvae and early juveniles of *N. oxleyana* based on collections of captive reared and wild specimens, and constitutes the first complete larval description for the genus. The study provides baseline information for research into the habitat associations, environmental tolerances, recruitment processes and population dynamics of *N. oxleyana*, for establishing successful conservation breeding and restocking programs if required, and contributes to a greater overall understanding of the conservation biology of this species.

Materials and Methods

Animal husbandry

Eggs and larvae were reared at the NSW Department of Industry and Investments' Port Stephens Fisheries Institute between November 2004 and May 2005. Techniques for breeding *N. oxleyana* are outlined in Knight *et al.* (2007). Briefly, broodstock were sourced from North Stradbroke Island, Qld. Six breeding pairs were housed separately in a recirculating, temperature controlled aquaria system. Adults were fed frozen bloodworms, live *Artemia* and mosquito wrigglers and spawned within acrylic spawning mops. Water quality, photoperiod (12 h light: 12 h dark) and water temperature (25 ± 1° C) for rearing eggs and larvae described in this study were based on average conditions experienced by wild populations in northern NSW during the spring/summer spawning period (Knight *et al.* 2007).

Spawning mops were checked regularly for eggs. Fertilised eggs were removed, counted and quickly assigned a developmental stage with the aid of a compound microscope. Embryos from the same parental stock and stage of development were grouped (termed a 'batch') and returned to the breeding tanks to hatch in floating petri dishes. Upon hatching, larvae from all parental stock were pooled, transferred to a flow-through fibreglass tank system (2.7 x 1.2 x 0.21 m) and housed in individual 2 litre cylindrical PVC holding containers at a mean ± S.E. stocking density of 19.6 ± 1.15 larvae per container. Water was gravity fed into each container and drained out the bottom through plankton mesh (0.5 mm), facilitating the exchange of water and removal of waste. Approximately 150 mL of live 'green water', dominated by cladocerans, rotifers and copepods (mean density: 16 zooplankters/mL), was added to each container daily. Juveniles were fed once daily with live newly hatched *Artemia* nauplii. On two brief occasions, larvae were also supplied with *Artemia* nauplii during periods of low natural zooplankton production.

Observations, measurements, terminology and counts

Embryonic development was documented by examining 60 aquaria-reared eggs sampled from 23 batches. Development was divided into three broad stages in eggs including 'Early' – fertilisation to closure of blastopore, 'Middle' – closure of blastopore to tail bud lifting off yolk, and 'Late' – tail bud lifting off yolk to time of hatching (Matarese and Sandknop 1984). Rate of embryonic development (hours post fertilisation) was calculated by systematically observing the timing of developmental events for a series of eggs from the same batch. Time of fertilisation of a batch was estimated by viewing video footage of spawning brood fish. Average time of each developmental event was rounded to the nearest half hour and expressed as hours post fertilisation (h). Eggs were discarded after one observation to avoid possible effects on development as a result of stress.

Between five and 10 larvae were collected daily for the first 14 days post hatching and then at 3-5 day intervals for observation and measurement. Where required, additional specimens were collected to assist in identifying the ranges of ages (days post hatching) and lengths at each developmental stage. Larval development was divided into three broad stages including 'Preflexion' – hatching to commencement of notochord flexion, 'Flexion' – commencement to completion of notochord flexion, and 'Postflexion'

– completion of notochord flexion to completion of squamation (Neira *et al.* 1998). Given that 0-day old larvae from all parental stock were pooled, the exact number of batches contributing to the study is not known. A total of 323 live larvae and juvenile fish from 97-170 batches were used to describe growth rates, colour in life, and the ontogeny of swimming and feeding (Table 1). Specimens were then preserved in 70% ethanol for subsequent description. Specimens preserved for 6 months shrunk an average of 0.23 ± 0.011 ± S.E. mm in body length (Range: 0.0–0.9 mm) or 3.08 ± 0.16% (Range: 0.0 – 12.2%). The equation for estimating the body length of live larval and juvenile fish (LBL) from preserved body length (BL) for fish of 2.75 to 15.3 mm BL is: LBL = 0.0782 + 1.0195BL (r^2 = 0.998, $P<0.001$, n = 186).

Larval morphological and meristic descriptions were primarily based on 37 captive reared, preserved specimens from 30-36 batches (Table 1). Data were also included from seven wild specimens collected in November 2005 from Evans Head, northeastern NSW (29° 04'S. 153° 23'E) using Perspex Quatrefoil traps set for 16 hours with a yellow cyalume light stick (Gilligan and Schiller 2003; Table 1). Wild-caught larvae were identified as percichthyids using the characteristics outlined in Leis and Trnski (2004), particularly their moderately deep body, relatively long gut, continuous dorsal fin, and counts of fin rays and myomeres. Wild larvae and juveniles were confirmed as *N. oxleyana* by comparison with captive reared specimens and meristic data for the species. Adult meristic counts are: **D** VI-VIII, 7-9 **A** III, 7-9 **P_1** 11-13 **P_2** I,5 **Vertebrae** 11-12+15-17 = 27-28 (Whitley 1940; Kuiter and Allen 1986; Kuiter *et al.* 1996; and Allen *et al.* 2002 and supplemented by x-rays of wild specimens housed in the Australian Museum fish collection).

Larval morphological definitions, measurements, and abbreviations follow Neira *et al.* (1998) and Leis and Carson-Ewart (2000). Eggs, larvae and juveniles were examined and measured under a dissecting microscope at magnifications from 8-50x. Precision of the measurements varied with magnification but ranged from 0.02 to 0.1 mm. Where larval morphometric values are given as a percentage, they are as a proportion of preserved body length unless otherwise indicated. All pigment described is external unless specified. Illustrations were prepared with a Zeiss SR microscope with an adjustable drawing tube. Photographs were taken with a Nikon Coolpix 5000 digital camera attached to a Leica Wild M3Z microscope.

All larvae examined (described and photographed specimens) are deposited in the fish collection at the Australian Museum (Registration numbers: I.44059-001 to -041 for the reared larvae and I.44060-001 to -007 for the wild larvae). Supplementary meristic data were obtained from x-rays of the following registered specimens held at the Australian Museum: *N. vittata* I.11230 (2 specimens), IA.6052 (1), IB.802-803 (5); *N. australis* I.1386 (1), I.16157-001 (1 paratype), I.19063-001 (4), I.30489-002 (3), I.40326-001 (3); *N. balstoni* I.13265 (1), I.13266 (1); *N. obscura* I.2265 (1), I.2266 (1); *N. oxleyana* I.17980-001 (4) , I.18953-003 (2), I.43422-002 (1), IA.3924 (3 paratypes), IB.523 (1 paratype); *N. variegata* I.25322-002 (1), I.25956-001 (3 paratypes).

Results

Embryonic Development

Early Development

Fertilised egg (0.0 h): Eggs average 1.02 ± 0.004 mm in diameter (Range: 0.98-1.10 mm), are transparent, spherical with a clear, smooth, chorion (0.006 mm thick) and lack filaments, but are demersal and slightly adhesive. The yolk is translucent, unpigmented, unsegmented, and homogeneous in texture and averages 0.87 ± 0.007 mm in diameter (Range: 0.80-0.95 mm). Between 1 and 39 yellow-coloured oil globules are located centrally above the yolk surface and vary considerably in size from 0.01-0.35 mm in diameter. Their low density causes the globules to orientate upwards. The perivitelline space is relatively narrow at the vegetal pole, varying from 0.02-0.09 mm in thickness. As in all teleosts, egg development is telolecithal and the cleavage pattern is meroblastic.

One cell stage – blastopore closure (1.0-16.0 h): An accumulation of cytoplasm in the animal pole gives rise to the first cell measuring 0.52 mm in diameter (Fig. 1a). Mitotic division (Fig. 1b-d) results in the formation of a blastoderm with a cellular appearance (Fig. 1e) that transforms to a granular and then uniform appearance

Table 1. Number, age and body length of *Nannoperca oxleyana* larvae and juveniles included in the study. Developmental terminology adapted from Neira *et al.* (1998) and Leis and Carson-Ewart (2000). LBL = live body length. BL = preserved body length.

Developmental stage		Days post hatch	Captive reared				Wild	
			Live specimens		Preserved specimens		Preserved Specimens	
			n	LBL (mm)	n	BL (mm)	n	BL (mm)
Preflexion	First hatch	0	25	2.80-3.40	1	3.05	0	
	Yolk-sac	1-12	78	3.40-4.65	5	3.65-4.30	0	
	Yolk absorbed	8-40	58	4.20-6.70	8	4.15-6.08	1	4.32
Flexion		21-61	17	6.35-7.70	6	6.00-6.80	3	6.81-7.18
Postflexion		43-118	50	6.40-10.30	13	6.08-9.51	2	7.30-9.02
Juvenile		70-188	95	9.60-20.60	4	8.87-13.75	1	12.69

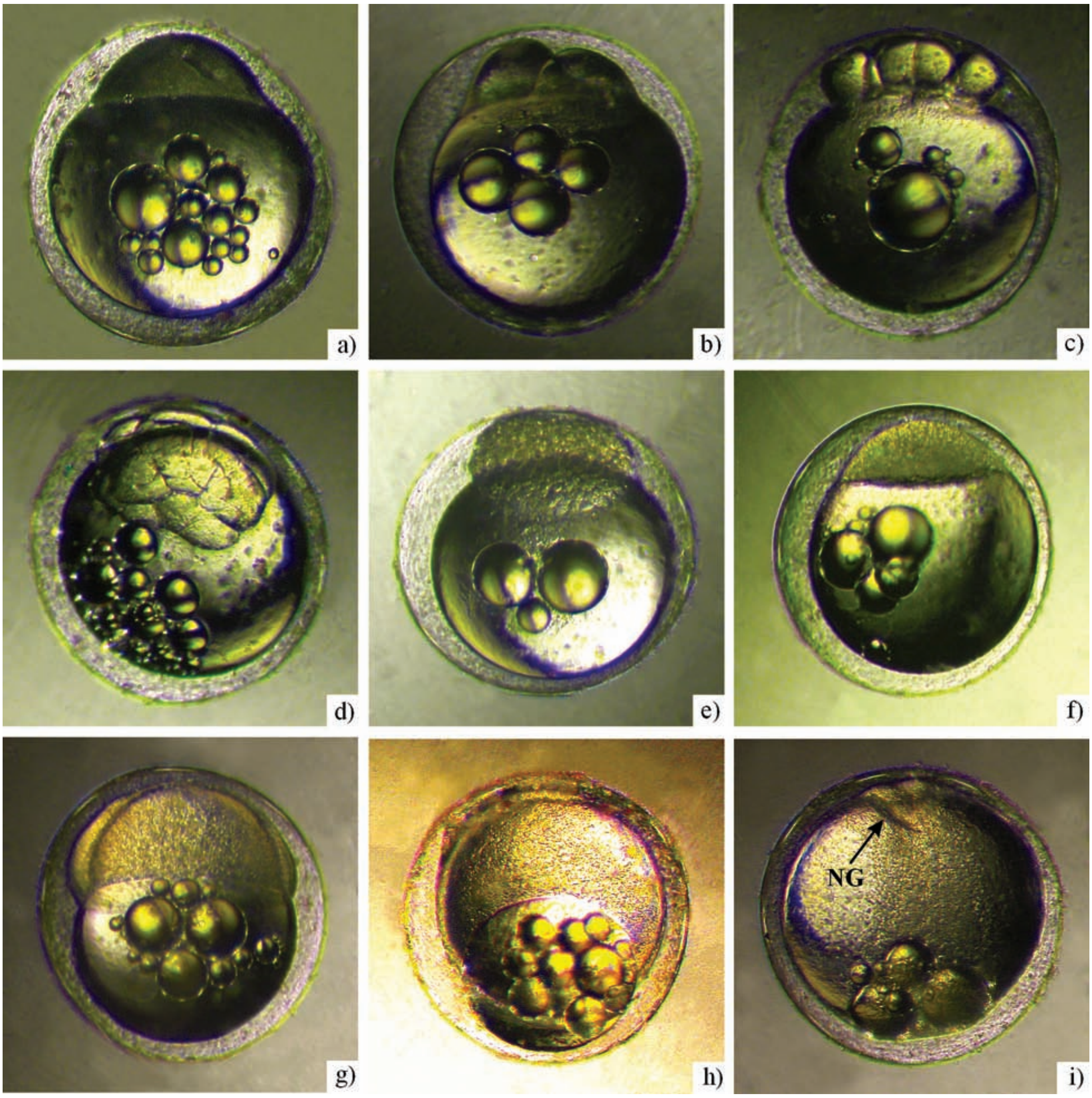

Figure 1. Early developmental stages of *Nannoperca oxleyana* eggs. a) one cell; b) two cells; c) eight cells; d) 16 cells; e) early blastoderm; f) blastoderm with granular appearance disappearing; g) epiboly; h) early yolk plug; i) gastrulation ending and neurulation commencing (NG, neural groove). Scale: Mean egg diameter is 1.02 mm.

(Fig. 1f) as the cells divide and reduce in size. Epiboly commences. The dome shaped blastocoel forms in the centre and the blastoderm becomes thinner and expands over the yolk mass towards the vegetal pole (Fig. 1g). The oil globules congregate in the yolk plug (Fig. 1h) prior to blastopore closure (Fig. 1i).

Middle Development

Early embryo – tail bud (16.0-23.5 h): The neural groove begins to develop along the embryonic axis just prior to the blastopore closure (Fig. 1i). Somites become differentiated, the cephalic region develops and the optic vesicles become visible (Fig. 2a). The early embryo reaches 190-200° around the yolk. Head width averages 0.24 mm, and the body reduces in width from 0.29-0.15 mm as it deepens, thereby creating a prominent neural ridge. Pigmentation begins with stellate melanophores averaging 0.01 mm in size becoming visible on the dorsal then ventral area of the yolk-sac at 0.02-0.12 mm intervals. Melanophores also appear in rows on the tail and trunk of the embryo stopping short of the optic vesicles.

Tail free (25.0 h): As the subcaudal fold forms, the tail bud begins to separate from the yolk. The embryo circles the yolk by approximately 260° and the myomeres are defined (Fig. 2b). The optic vesicles, which are not yet closed ventrally, contain a lens 0.05 mm in diameter (Fig. 2c). The oil globules begin to coalesce, the pectoral buds form as small bulges laterally and posterior of the cephalic region, (Fig. 2c), and the pericardial space is visible (Fig. 2d).

Late Development

Late embryo (28.0-50.0 h): As the embryo grows to encompass the yolk by 290°, approximately 25% of the body separates from the yolk and the notochord typically ends close to the oil globules (Fig. 2d). The otic vesicles become obvious. The circulatory system begins to function. The tail continues to separate from the yolk, the fin fold forms and the optic vesicles close ventrally (Fig. 2e). The embryo begins regular active body flexing and tail wriggling.

Prior to hatching, the embryo increases in length encompassing the yolk by at least 380° with the tail reaching past the optic capsule (Fig. 2f). The average measurements for head width, body width and body depth are 0.42 mm, 0.17 mm, and 0.15 mm, respectively. The body and yolk are heavily pigmented with small stellate melanophores. A row of large melanophores are also present on the dorsal and ventral midlines of the tail. Typically, one large and a number of smaller oil globules are centred at the anterior end of the yolk-sac.

Hatching (50.0h): Most embryos hatch within 30 minutes. Hatching begins with a slight protuberance in the chorion in the cephalic region of the embryo as the chorion becomes flaccid (Fig. 2g). Repeated flexing by the embryo enlarges the protuberance until the chorion finally bursts and the larva emerges usually head first (Fig. 2h and 2i). The oil globules coalesce into a single oil globule between one and three days post hatching.

Larval Development

General morphology (Table 2, Fig. 3a-d)

Larvae are elongate (body depth, BD 16-20%) at first hatch and until after the yolk is absorbed. The body becomes moderately deep in preflexion larvae from 5.0 mm BL and does not exceed 33% of BL. The body and head are moderately laterally compressed. There are 27-30 myomeres (9-14 preanal and 15-20 postanal); the anus is located under myomeres 9-10 until yolk-sac absorption, and under myomeres 11-14 in larger larvae. The gut is twisted in yolk-sac larvae and is coiled and triangular in preflexion larvae from 4.6 mm BL. The preanal length is 41-53% in preflexion larvae, and the gut becomes longer and elongate from the flexion stage (48-64%). The swim bladder is first visible in yolk-sac larvae from 4.2-4.3 mm BL; it is located

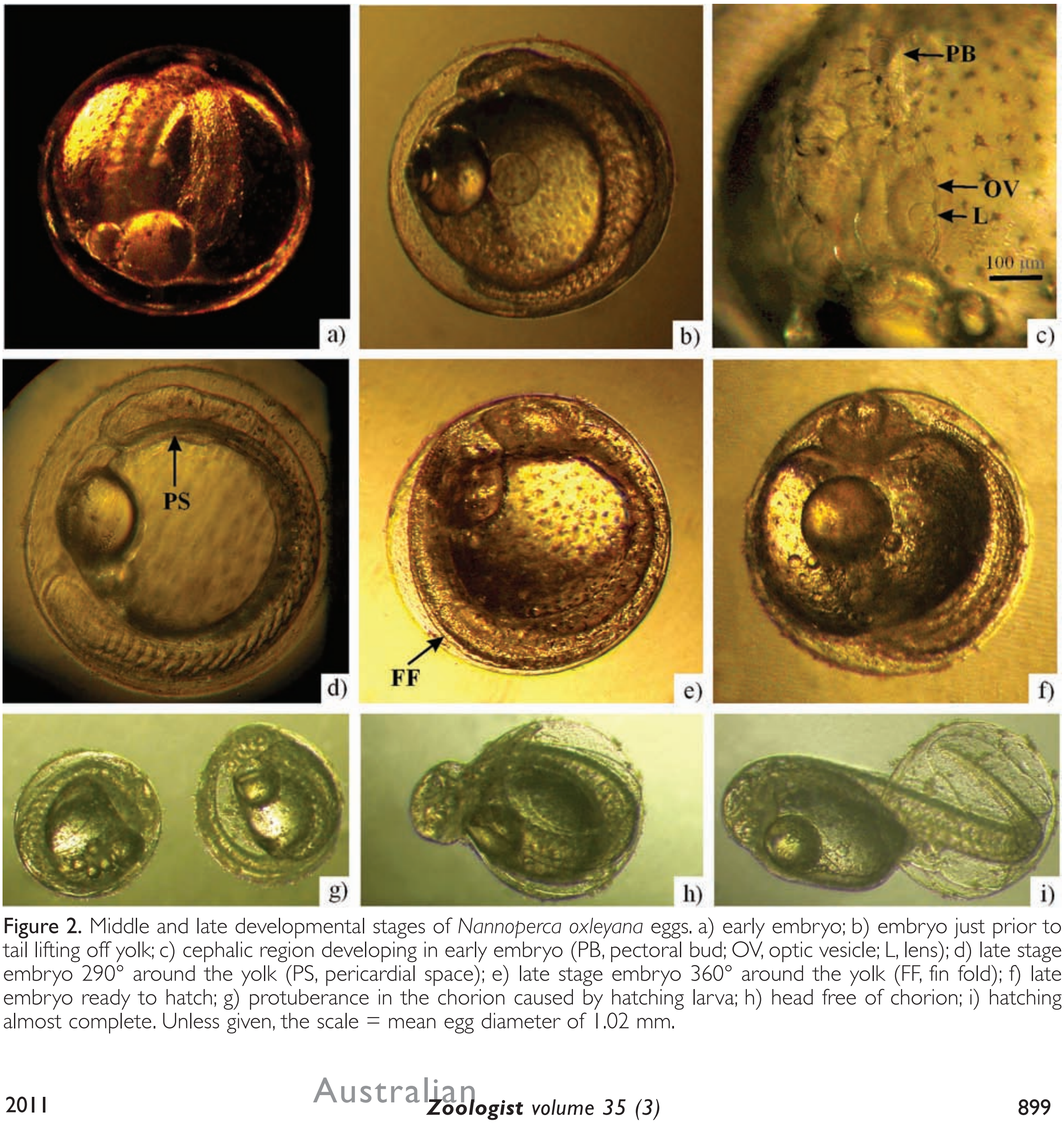

Figure 2. Middle and late developmental stages of *Nannoperca oxleyana* eggs. a) early embryo; b) embryo just prior to tail lifting off yolk; c) cephalic region developing in early embryo (PB, pectoral bud; OV, optic vesicle; L, lens); d) late stage embryo 290° around the yolk (PS, pericardial space); e) late stage embryo 360° around the yolk (FF, fin fold); f) late embryo ready to hatch; g) protuberance in the chorion caused by hatching larva; h) head free of chorion; i) hatching almost complete. Unless given, the scale = mean egg diameter of 1.02 mm.

Table 2. Morphometric data for preserved, captive reared and wild larvae and juveniles of *Nannoperca oxleyana*. Measurements are in mm. * indicates drawn specimen. $^{+}$ indicates photographed specimen.

Stage	Days post hatch	Total length	Body length	Preanal length	Pre dorsal -fin length	Body depth	Head length	Snout length	Eye diameter	Vent to anal fin length
First hatch	0	3.10	3.05	1.35		0.62	0.53	0.06	0.23	
Yolk	1	3.80	3.65	1.60		0.64	0.61	0.08	0.29	
Yolk*	2	3.90	3.75	1.60		0.62	0.70	0.10	0.31	
Yolk	11	4.25	4.15	1.80		0.71	0.92	0.12	0.41	
Yolk	5	4.40	4.25	1.77		0.70	0.81	0.11	0.34	
Yolk	9	4.40	4.30	1.89		0.76	0.96	0.11	0.43	
Preflex	10	4.30	4.15	1.70		0.65	0.87	0.10	0.35	
Preflex	Wild	4.44	4.32	1.92		0.78	1.00	0.16	0.42	
Preflex	17	4.70	4.60	2.10		0.81	1.00	0.12	0.49	
Preflex	17	4.90	4.90	2.25		0.87	1.09	0.14	0.50	
Preflex	30	5.15	5.05	2.45		1.02	1.21	0.22	0.50	
Preflex*	35	5.61	5.35	2.63		1.12	1.36	0.21	0.59	
Preflex	35	5.83	5.67	2.80		1.27	1.52	0.25	0.66	
Preflex	26	6.18	6.08	3.24	2.35	1.45	1.80	0.28	0.71	0.12
Preflex	36	6.08	6.08	3.00		1.46	1.58	0.28	0.71	0.00
Flexion	57	6.08	6.00	3.08	2.20	1.30	1.75	0.34	0.74	0.16
Flexion	30	6.12	6.08	2.92	2.40	1.35	1.65	0.25	0.71	0.16
Flexion	47	7.05	6.48	3.73	2.92	1.80	2.10	0.28	0.87	
Flexion	50	7.45	6.56	3.89	2.92	1.85	2.05	0.34	0.87	0.00
Flexion*	46	7.05	6.72	3.89	2.84	1.75	2.05	0.31	0.84	
Flexion	51	7.45	6.80	3.85	2.84	1.85	2.20	0.37	0.87	0.00
Flexion	Wild	6.97	6.81	3.48	2.68	1.64	1.96	0.32	0.76	0.00
Flexion	Wild	7.06	6.81	3.44	2.66	1.60	2.00	0.28	0.72	0.00
Flexion	Wild	7.39	7.18	3.74	2.80	1.76	2.00	0.36	0.84	0.00
Postflex	58	7.00	6.08	3.65	2.75	1.70	2.10	0.37	0.90	0.00
Postflex	61	7.61	6.32	3.65	3.00	1.75	2.30	0.37	0.99	0.16
Postflex	62	8.91	7.29	4.62	3.56	2.27	2.75	0.49	1.06	0.00
Postflex	Wild	8.38	7.30	4.40	3.32	2.16	2.48	0.48	0.92	0.00
Postflex	70	8.59	7.61	4.46	3.40	2.19	2.84	0.47	1.15	0.15
Postflex*	95	9.32	7.70	4.70	3.65	2.27	2.92	0.48	1.24	0.00
Postflex^{+}	56	9.64	8.02	4.94	3.65	2.51	2.92	0.40	1.20	0.00
Postflex	62	10.41	8.48	5.39	4.18	2.60	3.05	0.47	1.33	0.00
Postflex	63	10.54	8.48	5.46	3.98	2.70	3.40	0.68	1.49	0.06
Postflex	89	10.15	8.61	5.18	4.05	2.51	3.16	0.55	1.25	0.00
Postflex	63	10.92	9.00	5.65	4.24	2.80	3.30	0.62	1.46	0.06
Postflex	84	10.79	9.00	5.52	4.24	2.80	3.30	0.68	1.36	0.06
Postflex	Wild	10.86	9.02	5.40	4.15	2.84	3.28	0.56	1.20	0.00
Postflex	62	11.31	9.12	5.65	4.11	2.80	3.43	0.62	1.33	0.04
Postflex	61	11.57	9.51	5.78	4.50	2.95	3.55	0.58	1.43	0.06
Juvenile	70	10.79	8.87	5.40	4.11	2.65	3.25	0.56	1.40	0.09
Juvenile	81	11.05	9.00	5.59	4.40	2.95	3.60	0.59	1.55	0.09
Juvenile	118	14.65	12.00	7.45	5.67	3.72	4.40	0.78	1.72	0.25
Juvenile	Wild	15.36	12.69	7.76	5.81	4.15	4.56	0.83	1.66	0.08
Juvenile	105	16.77	13.75	8.61	6.30	4.24	4.86	0.80	1.84	0.12

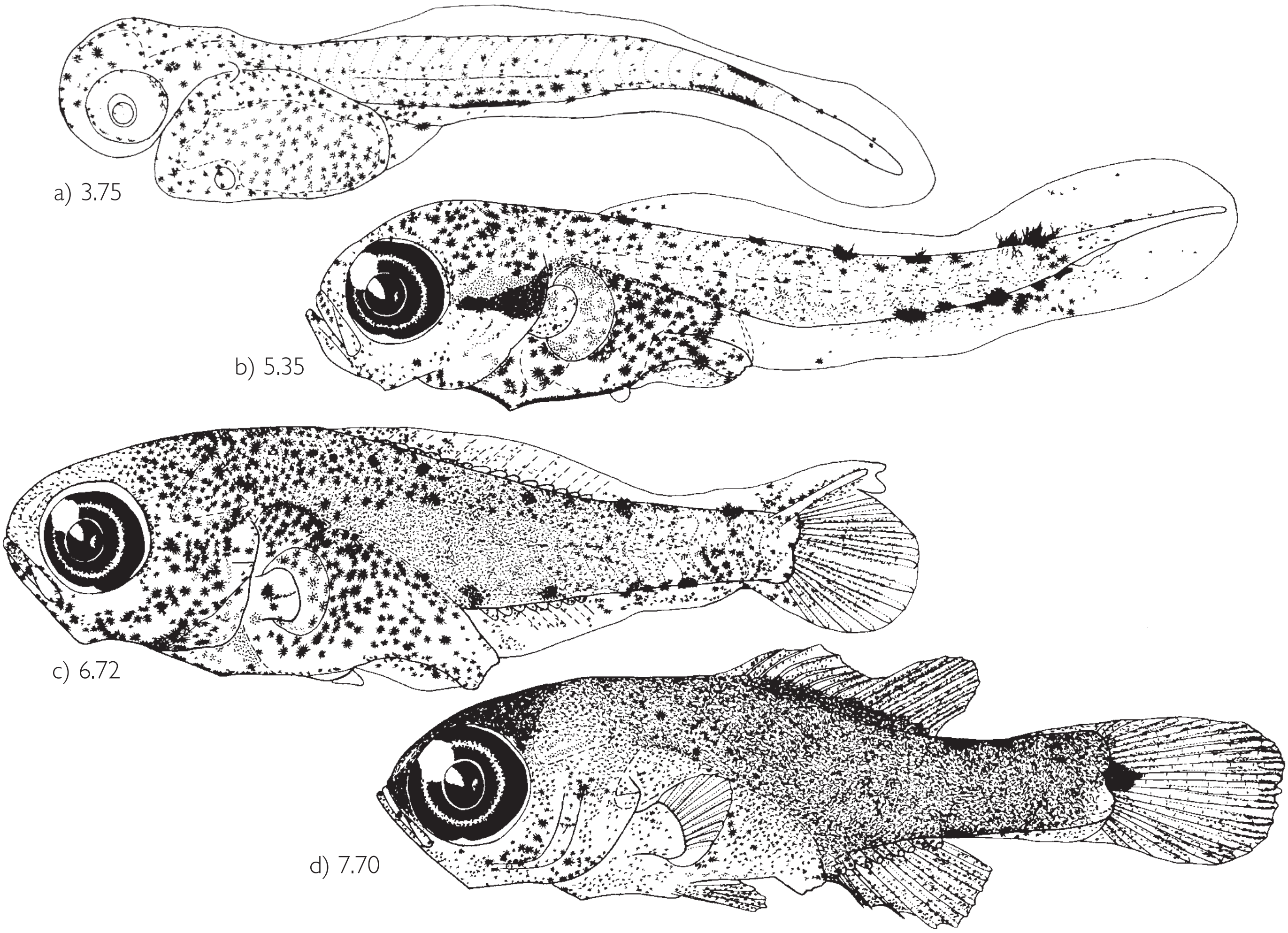

Figure 3. Larvae of *Nannoperca oxleyana.* a) yolk-sac larva; b) preflexion larva; c) flexion larva; d) postflexion larva. Larval body length (mm) is given in each figure.

over the midgut, always conspicuous and is usually large. The notochord tip is relatively elongate during notochord flexion. The round to slightly elongate head is initially small to moderate (head length, HL 17-22%), is moderate by the time yolk is fully absorbed and is large in postflexion larvae and juveniles (HL 35-40%). The snout is slightly convex, short and is never more than 50% of the eye diameter. The eye is round and large (eye diameter, ED 36-49% HL). The small to moderate mouth reaches midway between the anterior margin of the eye and the pupil. Teeth were not apparent in any of the larvae or juveniles examined. The nasal pit forms by the flexion stage, and roofs over in juveniles, by 8.9-9.5 mm. Larval head spination is absent. A weak opercular spine is present from the flexion stage.

Meristics (Table 3; Fig. 3a-d)

Dorsal- and anal-fin anlagen form by the commencement of notochord flexion. Bases and incipient rays of the dorsal and anal fins develop during notochord flexion, and the elements are ossified in postflexion larvae from 6.3 mm BL. The full complement of dorsal and anal elements is present by 6.3-8.5 mm BL. Anal-fin counts in reared larvae may be outside published ranges; this appears to be an artifact of rearing. The pectoral fins begin to form incipient rays in postflexion larvae from 7.3 mm BL, the rays ossify from dorsal to ventral and all are present by 8.5 mm BL. Pelvic-fin buds appear in preflexion larvae from 5.4 mm BL, the membrane forms during flexion, and ossification of the elements commences in postflexion larvae from 6.3 mm BL. The pelvic-fin elements are all ossified by 7.6 mm BL. Caudal-fin anlage appear in preflexion larvae from 5.4 mm BL, and rays commence ossification in flexion larvae. All primary caudal-fin rays are ossified in postflexion larvae by 7.6 mm BL. The smallest and largest larvae undergoing notochord flexion were 6.0 mm and 7.2 mm BL.

Squamation

Squamation does not begin until postflexion larvae attain 7.5 mm BL. A single row of clear, ctenoid scales initially appear along the notochord while the median fins are still developing. The scales spread along the body laterally, then dorsally and ventrally. The last regions to become scaled are the opercula and ventrally around the pelvic fins, followed by the nape, and finally the hypural bones. The smallest fully scaled juvenile measured 9.2 mm BL and all specimens greater than 10.3 mm had completed squamation.

Pigment (Fig. 3a-d)

Larvae are moderately pigmented at first hatch and become heavily pigmented by the time the yolk is absorbed. Melanophores are concentrated on the dorsal and ventral midlines of the tail, and midlateral surface of the head and trunk. Small expanded melanophores are present at the tip of the upper and lower jaws, ventrally along the lower jaw,

Table 3. Meristic data for preserved, captive reared and wild larvae and juveniles of *Nannoperca oxleyana*. Incipient elements are given in parentheses. Myomeres are divided into pre- and post-anal counts (Pre and Post, respectively). * indicates drawn specimens. + indicates photographed specimen.

Stage	Days post hatch	BL	Fin-ray counts					Myomeres		
		(mm)	Dorsal	Anal	Pectoral	Pelvic	Caudal	Pre	Post	Total
First hatch	0	3.05			buds			9	19	28
Yolk	1	3.65			buds			9	20	29
Yolk*	2	3.75			buds			9	19	28
Yolk	11	4.15			0			10	18	28
Yolk	5	4.25			0			10	19	29
Yolk	9	4.30			0			10	18	28
Preflex	10	4.15			0			11	18	29
Preflex	Wild	4.32			0			9	19	28
Preflex	17	4.60			0			11	17	28
Preflex	17	4.90			0			13	17	30
Preflex	30	5.05			0			12	16	28
Preflex*	35	5.35			0	weak fin base	anlage	13	15	28
Preflex	35	5.67			0	fin base	fin base	13	16	29
Preflex	26	6.08	13 bases	7 bases	0	fin base	(2+2)	13	16	29
Preflex	36	6.08			0	fin base	(1)	13	15	28
Flexion	57	6.00	anlage	anlage	0	fin base	2+2	11	16	27
Flexion	30	6.08	anlage	anlage	0	fin base	2+3	11	17	28
Flexion	47	6.48	(VII, 7)	(II, 7)	0	membrane	8+7	12	16	28
Flexion	50	6.56	(VI, 7)	(II, 7)	0	membrane	9+8	14	16	30
Flexion*	46	6.72	(VI, 8)	(6)	0	membrane	7(1)+7	12	16	28
Flexion	51	6.80	(VI, 8)	(II, 7)	0	incipient rays	8+8	13	15	28
Flexion	Wild	6.81	(VI, 6)	(I, 5)	0	0	2(5)+5(2)	11	17	28
Flexion	Wild	6.81	(VI, 6)	(I, 6)	0	0	2(4)+3(2)	11	18	29
Flexion	Wild	7.18	VI, 8	II, 9	0	0	8+7	12	17	29
Postflex	58	6.08	(VII), 5(3)	(I), 7	0	membrane	8+8	13	15	28
Postflex	61	6.32	VII, 7	II, 7	0	I, 3(2)	9+8	12	17	29
Postflex	62	7.29	VII, 7	II, 8	(2)	I, 5	9+8			
Postflex	Wild	7.30	VI, 8	III, 8	0	(3)	8(1)+8	13	16	29
Postflex	70	7.61	VII, 8	II, 7	7	I, 5	9+8			
Postflex*	95	7.70	VII, 8	III, 7	10	I, 5	9+8			
Postflex+	56	8.02	VI(I), 7	II, 8	9	I, 5	9+8			
Postflex	62	8.48	VII, 8	III, 7	8	I, 5	9+8			
Postflex	63	8.48	VI, 9	II, 7	12	I, 5	9+8			
Postflex	89	8.61	VII, 8	II, 7	12	I, 5	9+8			
Postflex	63	9.00	VII, 8	III, 7	12 or 13	I, 5	9+8			
Postflex	84	9.00	VII, 8	III, 8	11	I, 5	9+8			
Postflex	Wild	9.02	VI, 8	III, 8	7	I, 5	9+8			
Postflex	62	9.12	VII, 7	III, 8	10	I, 5	9+8			
Postflex	61	9.51	VI, 9	III, 7	12	I, 5	9+8			
Juvenile	70	8.87	VII, 8	III, 7	10 or 11	I, 5	9+8			
Juvenile	81	9.00	VI, 8	II, 7	12	I, 5	9+8			
Juvenile	118	12.00	VI, 8	I, 7	11 or 12	I, 5	9+8			
Juvenile	Wild	12.69	VII, 9	III, 8	11	I, 5	9+8			
Juvenile	105	13.75	VII, 8	II, 7	11	I, 5	9+8			

dorsally on the head and over the operculum. The nasal pit is unpigmented. Internal melanophores are present along the roof of the mouth, and posterior to the eye below the mid- and hindbrain. A matching horizontal band of external melanophores is present on the operculum in line with the eye until the early postflexion stage. The eye is initially unpigmented, but becomes pigmented during yolk absorption (by 4.2 mm BL).

Small melanophores are present along the ventral midline of the gut, from anterior of the cleithral symphysis to the anus. Additional melanophores cover the remainder of the gut except there may be an unpigmented region immediately above the pelvic-fin base in preflexion and flexion larvae.

Five to seven large, expanded melanophores are present along the dorsal midline of the trunk (1 or 2 melanophores) and tail (4 or 5), from the nape to the caudal peduncle; these are apparent in preflexion larvae by the time the yolk is absorbed. A series of melanophores is present along the lateral midline of the trunk and tail, commencing at the swim bladder and extending to the posterior end of the dorsal and anal fins. In postflexion larvae, this series extends onto the anterior third of the caudal peduncle. Up to six expanded melanophores are present along the ventral midline of the tail, from above the anus to the caudal peduncle. Between one and three of these expanded melanophores occur along the anal-fin base. A small melanophore is occasionally present in early postflexion larvae at the base of ventral primary caudal-fin rays 1-2. Most of the expanded melanophores on the dorsal and ventral midlines migrate internally and become difficult to distinguish by the juvenile stage. Only two dark patches remain in juveniles, one each on the dorsal and ventral margins of the caudal peduncle immediately posterior to the dorsal and anal fins, respectively. Internal melanophores are present over the swim bladder, the mid- and hindgut, and may be present along the notochord. The external and internal pigment series thus give the impression of a line of heavy pigment from the tip of the snout, across the head and trunk to the tail. A large black dot, characteristic of the species, begins to form at the base of the caudal fin as a tight group of small black melanophores in flexion larvae and is completely developed in postflexion larvae.

By the juvenile stage, additional melanophores develop laterally on the head and body, and all the fins become pigmented. Melanophore coverage is lightest ventrally on the head and gut. Three broad vertical bands become apparent dorsally on the nape, below the centre of the spinous dorsal fin and below the centre of the soft dorsal fin in juvenile fish from 13.5 mm in length.

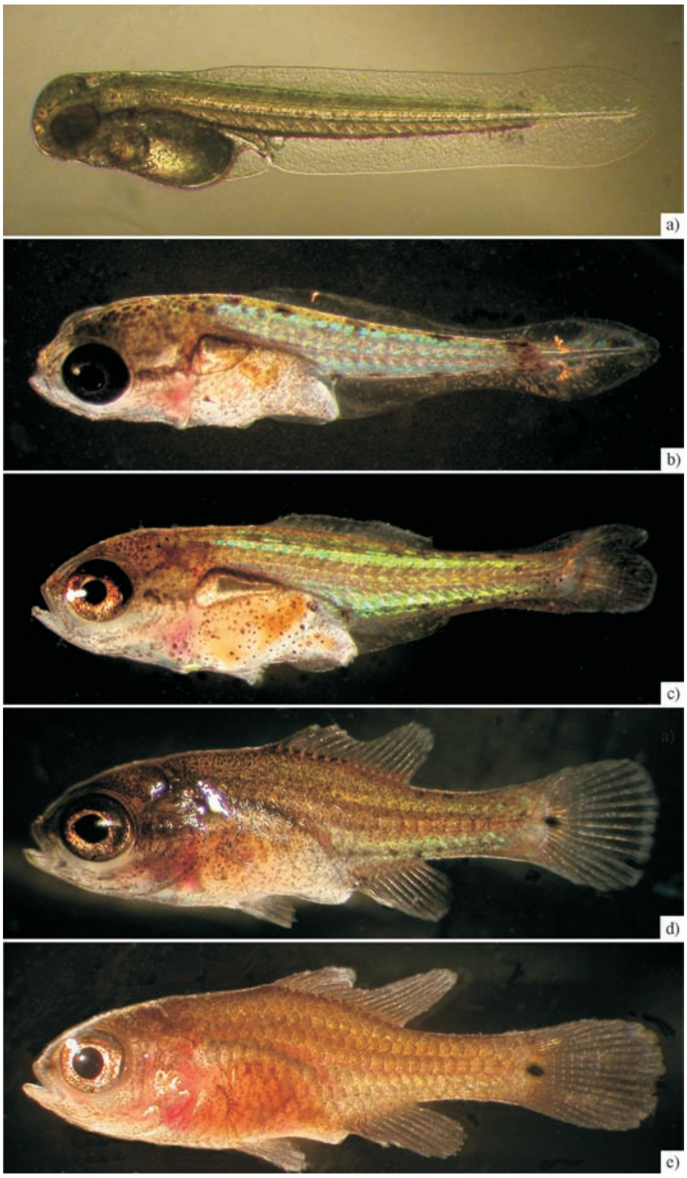

Figure 4. Micrographs of live larvae and a juvenile of *Nannoperca oxleyana*. a) yolk-sac larva, 3 days post hatch, 4.0 mm BL; b) late preflexion larva, 25 days post hatch, 6.0 mm BL; c) late flexion larva, 52 days post hatch, 7.6 mm BL; d) late postflexion larva, 56 days post hatch, 8.6 mm; e) juvenile, 136 days post hatch, 12.9 mm BL.

Colour in life

At hatching, live larvae are transparent. With the exception of the yolk, larvae turn pale green within two days of hatching (Fig. 4a). A black line also forms ventrally along the tail, running above the yolk to the posterior of the eye. Pigmentation develops in the eyes and branching chromatophores appear, occurring dorsally and ventrally on the trunk and tail. At four days post hatching (3.9-4.3 mm LBL), the eyes become fully pigmented taking on a speckled black and gold appearance. The black line along the tail fades in preflexion larvae, remaining only above the swim bladder (Fig. 4b), and is absent in juveniles. The branching chromatophores also gradually dissipate until they are no longer visible in flexion larvae (Fig. 4c). The nape and dorsal area of the head of preflexion larvae become light brown, followed by the trunk and tail in flexion and postflexion larvae (Fig. 4c and d). Iridescent golden scales develop in postflexion larvae on the operculum, laterally on the stomach and ventrally around the pelvic fins. Fully scaled juveniles are typically light brown laterally, darker dorsally and have a silvery-white belly (Fig. 4e). The iridescent scales remain while all other scales have black margins with small peppery melanophores. Sparse red pigmentation also occurs in the skin on the trunk of some postflexion and juvenile fish.

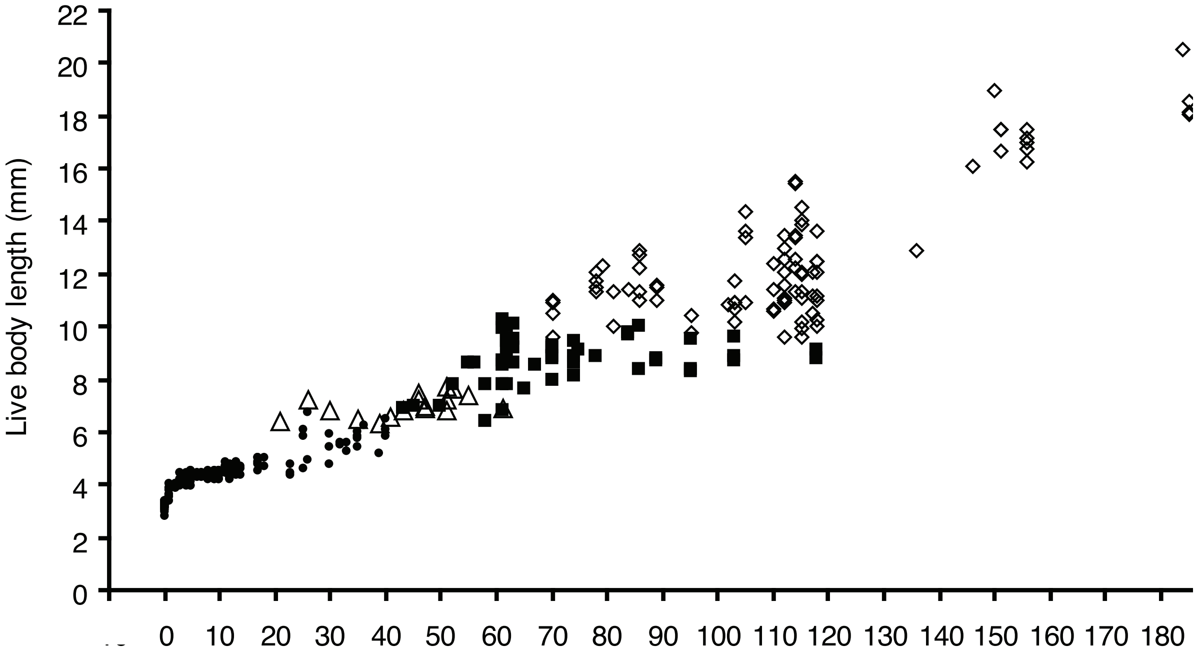

Figure 5. Growth and development of captive reared *Nannoperca oxleyana* larvae and juveniles over 188 days (n = 323). Development includes preflexion ●, flexion Δ, postflexion ■, and juvenile ◊ stages.

Growth

The growth of 323 live, captive reared fish over a 6-month period is depicted in figure 5. Initial growth was relatively rapid. Newly hatched larvae (2.8-3.4 mm LBL, mean: 3.17 ± 0.027 mm LBL), increased in mean length by 0.49 mm in the first 24 hours post hatching. Mean growth rates decreased over the following three days to 0.28 mm, 0.21 mm and 0.09 mm, respectively and then proceeded to plateau at a mean daily increase of only 0.02 mm in larvae 5-10 days post hatching. Growth rates returned to a mean daily increase of 0.23 mm in 14-day-old larvae (LBL: 4.56 ± 0.075 mm). Beyond this period, growth rates became more variable. The mean monthly LBL of fish of 1 to 6-months of age were 5.74 ± 0.140 mm, 8.78 ± 0.252 mm, 9.80 ± 0.401 mm, 11.46 ± 0.330 mm, 17.24 ± 0.259 mm and 18.65 ± 0.255 mm, respectively.

There was increased variation in length as fish aged, with the coefficient of variation ranging from 2 to 4% up to 17 days post hatching, to as high as 19% for older larvae. Variation in the distribution of residuals from the linear regression of LBL at age violated the assumption of homogeneous variances. This problem was best resolved by a log 10 transformation of body length (Log_{10} LBL), with the residual plots showing no increase in variance with age (A). Hence, larvae and juveniles showed logarithmic growth for the 6-month period, which is best described by the equation: $\mathrm{Log}_{10}\ \mathrm{LBL} = 0.6043 + 0.0042A$ ($r^2 = 0.917$, $P<0.001$, $n = 323$).

Body length was a more precise measure of larval development than age as there was less overlap of the major developmental stages with body length than with age (Fig. 5; Table 1). Newly hatched larvae took up to 40 days to reach the end of the preflexion stage at a maximum LBL of 6.70 mm. The flexion stage was almost completely overlapped by the preflexion and postflexion stages in terms of body length and age. Flexion larvae ranged in size from 6.35-7.70 mm LBL and in age from 21-61 days post hatching. The smallest and youngest postflexion larvae were 6.40 mm LBL and 43 days old, respectively, and the largest and oldest were 10.30 mm LBL and 118 days old. However, the smallest juvenile studied measured 9.6 mm LBL and the youngest was 70 days post hatching.

Feeding and Swimming Ontogeny

Given that larval length remained relatively constant between the ages of 5 and 10 days post hatching, age was used to describe the ontogeny of larval feeding and swimming. The yolk sustained larvae for the first four days post hatching. During this time, the head separated from the yolk, the eyes became pigmented, the jaw developed, detached from the yolk and opened, and the gut formed in the place of the yolk-sac.

Newly hatched larvae were unable to swim or maintain buoyancy and typically remained motionless on the substrate. When disturbed, larvae briefly kicked sideways off the substrate in a spiralling motion then relaxed and sank. As the pectoral fins developed, larvae became more active, but still swam in a spiralling motion in a lateral orientation. Swim bladder inflation commenced 4 days post hatching, and 24 hours later all larvae were able to maintain buoyancy in the water column and swim freely in a dorsal-ventral orientation. At this time, larvae were 3.90-4.55 mm LBL and were often seen near the water surface presumably searching for food.

As the larvae developed, the yolk-sac was absorbed and gradually decreased in size (Fig. 6). Yolk-sac length decreased by a mean of 62% between 4 and 5 day old larvae. This coincided with increased development of the mouth and the commencement of exogenous feeding, as evidenced by peristalsis, the presence of green algae in the stomach and intestines, and waste

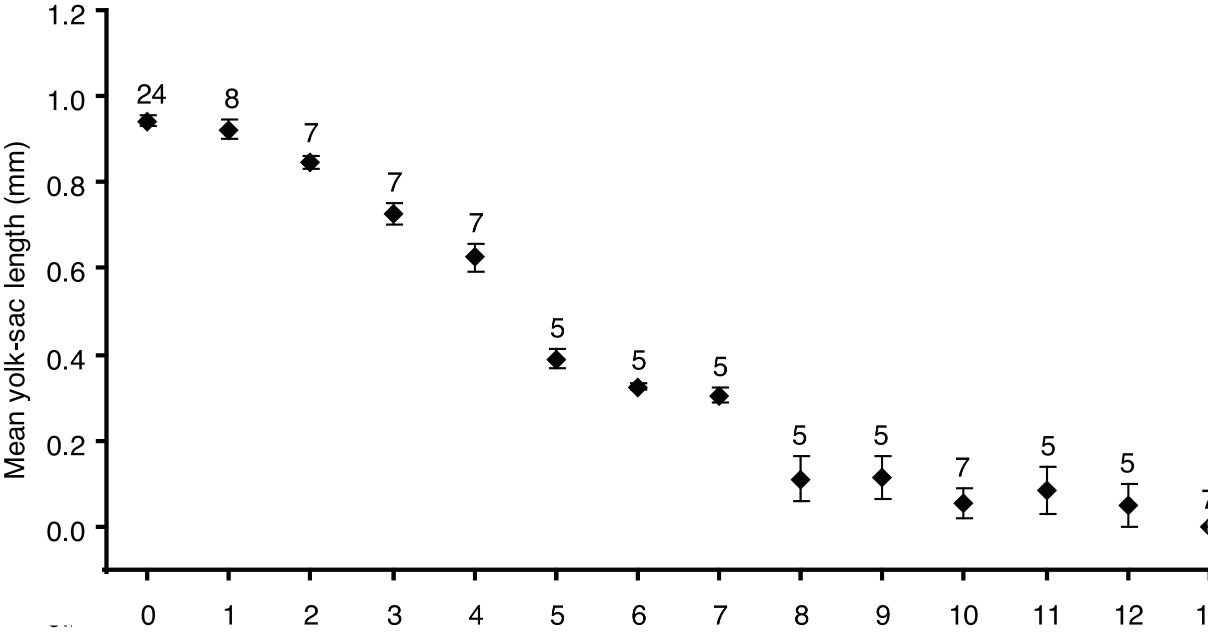

Figure 6. Plot of mean ± S.E. yolk-sac length versus larval age (n = 107). Sample sizes for each age class are given.

material in the lower intestine and anus. Complete yolk-sac absorption was first observed in eight day old larvae, and had occurred in all larvae greater than 12 days of age (Fig. 6).

Discussion

The eggs of *N. oxleyana* are typical of those of most teleosts, being telolecithal and spherical, having a smooth chorion and translucent, homogeneous yolk, displaying meroblastic cell division and following the general pattern of embryogenesis (Blaxter 1988; Kendall *et al.* 1984). In addition, the eggs are demersal as in most freshwater fishes and percichthyids (Johnson 1984; Kendall *et al.* 1984; Harris and Rowland 1996; Pusey *et al.* 2004), and are morphologically similar to those described for other *Nannoperca* species (Shipway 1949; Llewellyn 1974; Morgan and Beatty 2000). A lack of filaments on the chorion is a further feature characteristic of Australian percichthyids (Harris and Rowland 1996; Pusey *et al.* 2004).

Within the Percichthyidae, the size of *N. oxleyana* eggs (0.98-1.10 mm) is similar or slightly smaller than those described for *N. australis* (1.16-1.35mm), *N. vittata* (1.1-1.2 mm), the two catadromous *Macquaria* species (0.9-1.5 mm), and *Bostockia porosa* (1.4-1.8 mm), and is much smaller than those of *Maccullochella* spp. and the two freshwater *Macquaria* species (2.5-4.2 mm) (Shipway 1949; Lake 1967; Llewellyn 1974; Cadwallader and Rogan 1977; Pen and Potter 1990; Harris and Rowland 1996; Neira *et al.* 1998; Morgan and Beatty 2000; Pusey *et al.* 2004; Trnski *et al.* 2005).

Development of the eggs was very similar to that of *N. australis*, although the development rate was faster in *N. oxleyana* (i.e. early development 16 hrs vs 27 hrs, middle development 25 hrs vs 39.5 hrs, late development 50 hrs vs 74 hrs). The shorter embryonic period may have been at least in part a result of different rearing temperatures, with *N. australis* reared at lower temperatures ranging between 15.8 and 25.3° C (Llewellyn 1974). Eggs of *N. oxleyana* spawned and reared at 16° C took approximately 144 hrs to hatch (J. Knight, unpublished data).

Throughout its distributional range, *N. oxleyana* is known to co-occur with 16 species of fishes belonging to the families Eleotridae, Melanotaeniidae, Pseudomugilidae, Galaxiidae, Ambassidae, Plotosidae, Percichthyidae, Anguillidae, and Poeciliidae (Arthington and Marshall 1993; Arthington 1996; Knight 2000, in press). Of these, the two anguillids spawn in the deep ocean (Allen *et al.* 2002), the poeciliid *Gambusia holbrooki* is viviparous (live bearers) (Milton and Arthington 1983), *Galaxias maculatus* spawns eggs that develop out of water on moist, riparian vegetation (Pollard 1971; Allen *et al.* 2002), and the percichthyid *Macquaria novemaculeata* is catadromous, breeding in estuarine areas in winter (Harris 1986; Trnski *et al.* 2005). Eggs of *N. oxleyana* can be distinguished from those of the remaining sympatric species through a combination of egg shape, size and the absence of filaments (Table 4).

Morphology of the larvae of *N. oxleyana* is similar to that of other percichthyids described from Australia (Brown and Neira 1998, Trnski *et al.* 2005). There is an absence of larval specialisations. Head spination is limited to an opercular spine whereas most other described percichthyids also develop weak to moderate preopercular spines during, or shortly after, notochord flexion. *Nannoperca oxleyana* can be distinguished from other percichthyids by a combination of morphometrics, meristics and pigmentation pattern. In particular, the ratio of ED:SnL is greater than 200% throughout development (191% in only one wild specimen in the described series), which is higher than for other described percichthyids.

Table 4. Major distinguishing characteristics of the eggs of *Nannoperca oxleyana* and 11 sympatric indigenous species. Data sourced from Lake (1967); Anderson *et al.* (1971); Llewellyn (1971); Auty (1978); Howe (1987); Koehn and O'Connor (1990); Semple (1986, 1991); and Pusey *et al.* (2004).

Family	Genus/Species	Shape	Size (mm)	Filaments
Ambassidae	*Ambassis agassizii*	spherical	0.6-0.7	no
Eleotridae	*Gobiomorphus australis*	unknown	unknown	unknown
	Hypseleotris compressa	slightly pear shaped	0.26-0.28 × 0.30-0.32	no
	Hypseleotris galii	oblong	0.91 × 0.62	no
	Philypnodon grandiceps	elongate to elliptical	1.5-2.2 × 0.7-0.9	no
	Philypnodon macrostomus	teardrop-shaped	unknown	no
Melanotaeniidae	*Melenotaenia duboulayi*	spherical	0.88-1.5	yes
	Rhadinocentrus ornatus	spherical	1.2-1.35	yes
Percichthyidae	*Nannoperca oxleyana*	spherical	0.98-1.10	no
Plotosidae	*Tandanus tandanus*	spherical	3.1-3.4	no
Pseudomugilidae	*Pseudomugil mellis*	spherical	1.26-1.64	yes
	Pseudomugil signifer	spherical	1.13-1.8	yes

In some respects, the larvae of *N. oxleyana* more closely resemble those of other small to medium sized Australian percichthyids than the larger fishes of the genus *Maccullochella*. The larval lengths of *N. oxleyana* at hatching are comparable to those recorded for *N. australis* (3.2-3.9 mm), *N. vittata* (3.0-3.2 mm), *Macquaria ambigua* (2.5-3.4 mm) and *M. novemaculeata* (2.5-3.5 mm), but are much smaller than those of *Maccullochella* spp. (5.0-9.0 mm), which hatch with a massive yolk (Shipway 1949; Lake 1967; Llewellyn 1974; Harris and Rowland 1996; Neira *et al.* 1998; Morgan and Beatty 2000; Pusey *et al.* 2004; Trnski *et al.* 2005). The larvae of species with similar lengths at hatching are also poorly developed at this time with unpigmented eyes and unformed mouths, while their lengths and ages at the commencement of exogenous feeding are also similar (lengths of 3.9-5.4 mm; ages of 3-8 days). Furthermore, in *N. oxleyana*, *N. australis*, *N. vittata*, *N. balstoni*, *Macquaria ambigua*, and *Macquaria novemaculeata* the yolk is fully absorbed prior to notochord flexion, whereas in *Maccullochella peelii* and *Maccullochella macquariensis* the remnants of the yolk-sac are still visible in postflexion larvae (Llewellyn 1974; Harris and Rowland 1996; Rowland 1996; Brown and Neira 1998; Gill and Morgan 1998; Morgan and Beatty 2000; Pusey *et al.* 2004; Trnski *et al.* 2005).

Examination of larvae of *N. australis*, *N. obscura* and *N. variegata* revealed morphological and developmental similarities between these species and *N. oxleyana* (T. Trnski, unpublished data). All species have melanophores distributed over the entire head and body with an internal line of pigment from the posterior of the eye to dorsally on the swim bladder and gut. *Nannoperca australis* and *N. obscura* also have 4-6 small patches of darker pigment on the dorsal midline of the trunk and tail, and the ventral midline of the tail, though these patches are absent in *N. variegata.* Although not sympatric, larvae of *N. oxleyana* can be distinguished from other pygmy perch species by meristics: *Nannoperca oxleyana* (D VI-VIII, 7-9; A III, 7-9; Vert 11-12+15-17=27-28), *Nannoperca australis* (D VI-IX, 7-10; A III, 6-9; Vert 12-13+16-18=29-30), *Nannatherina balstoni* (D VII-IX, 9-11; A III, 8-10; Vert 14+18=32), *Nannoperca obscura* (D VIII-IX, 7-9; A III, 6-8; Vert 12+18=30), *Nannoperca variegata* (D VII-IX, 9-10; A III, 8-9; Vert 12-14+17-19=30-32) and *Nannoperca vittata* (D VII-IX, 8-11; A III, 6-9; Vert 11-13+17-18=29-31) (Whitley 1940; Kuiter and Allen 1986; Kuiter *et al*. 1996; Allen *et al*. 2002; and supplemented by x-rays of wild specimens housed in the Australian Museum fish collection).

Nannatherina balstoni is the only other pygmy perch for which the larvae have been fully described (Gill and Morgan 1998). This species obtains a larger maximum size than *N. oxleyana* of approximately 90 mm. Ranges of body lengths for each stage of larval development of *N. balstoni* are: preflexion 4.9 – 6.6 mm, flexion 6.6-11.1 mm, postflexion 10.9-14.5 mm, and juveniles 14.1-23.2 mm. Apart from that of preflexion larvae, these ranges were larger at an equivalent stage than those for *N. oxleyana*. Squamation also commenced at a larger size in *N. balstoni* at 13 mm.

Melanophore distribution in the wild *N. oxleyana* larvae used in this description was sparser and lighter compared with the reared larvae. It is widely accepted that captive reared larvae are often heavier and have deeper bodies than wild caught specimens, display greater meristic variation and are frequently more heavily pigmented (Hunter 1984). Similarly, wild *N. oxleyana* larvae developed their meristic complements at a larger size than the reared series. These differences may be due to rearing conditions and also time of capture of the specimens.

It appears that four to five days post hatching represents an important period in the development of *N. oxleyana*. A combination of morphological, physiological and behavioural developments enabled the larvae to actively search for food and signalled the commencement of exogenous feeding. A similar timing and pattern of development has been observed for *N.*

australis, *N. vittata*, *M. ambigua* and *M. novemaculeata* (Llewellyn 1974; Battaglene and Talbot 1990; Rowland 1996; Morgan and Beatty 2000). Endogenous feeding was also still apparent at this time and growth rates proceeded to plateau over the following five to seven days as the last of the yolk was absorbed and larvae switched to full exogenous feeding. Trends in mortality rates may also reflect the transition from endogenous to exogenous feeding. On two occasions it was noted that the mean survival of 29 batches of larvae declined from 60.1% for larvae between the ages of 6 and 10 days to 10.4% for 11-14 day old larvae (J. Knight, unpublished data). Indeed, this transitional period is often one of high mortality (Blaxter 1969).

In this study, length proved to be a better measure of ontogenetic stage than age, at least after yolk-sac absorption. Ontogenetic states are often reached at uniform sizes, regardless of the time taken to achieve them (Fuiman *et al.* 1998). Development rates are often influenced by environmental variables such as water quality, temperature, lighting regimes and food resources (Blaxter 1969; Fuiman *et al.* 1998). The rate of ontogenesis of eggs and larvae documented in this study may therefore simply reflect the rearing conditions in the laboratory. However, the potentially confounding effects of artificial conditions were minimised by utilising water with physico-chemical properties similar to that of waters supporting wild populations, by adopting the average photoperiod and water temperatures experienced by wild populations during the breeding season, and by providing a diet of zooplankton species recorded in the diets of wild larvae, juvenile and adult pygmy perch (Pen and Potter 1991; Pen *et al.* 1993; Arthington and Marshall 1993; Humphries 1995; Morgan *et al.* 1995; Arthington 1996; Gill and Morgan 1998). Research into the age and growth of *N. oxleyana* in the wild may assist in assessing if development and growth rates in captivity differ from those of wild fish.

Nannoperca oxleyana is one of Australia's most endangered species and is the most threatened fish species to inhabit the coastal wallum ecosystems of mid-eastern Australia (NSW Department of Primary Industries 2005). Although the eggs of this species lacked filaments, in this study their slight adhesiveness facilitated attachment to the spawning mops until hatching. It is plausible that eggs spawned in the wild would attach to submerged riparian and aquatic vegetation in a similar way (Knight *et al*. 2007). Indeed, juveniles and adults occupy dense macrophyte beds and submerged riparian vegetation throughout the year (Knight and Arthington 2008). All of the wild larvae described in this study were also captured in these habitats. This habitat may therefore provide *N. oxleyana* with a refuge from predators and strong currents, and suitable feeding grounds throughout its entire life cycle. Management practices, such as groundwater extraction, fire management, agriculture, dredging, channelisation, and urban development, that reduce or degrade available spawning, nursery and adult habitat pose a serious threat to this species (Pusey *et al*. 2004; NSW Department of Primary Industries 2005). The conservation and recovery of *N. oxleyana* therefore depends largely on the maintenance of this habitat within the coastal, wallum ecosystems of mid-eastern Australia.

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